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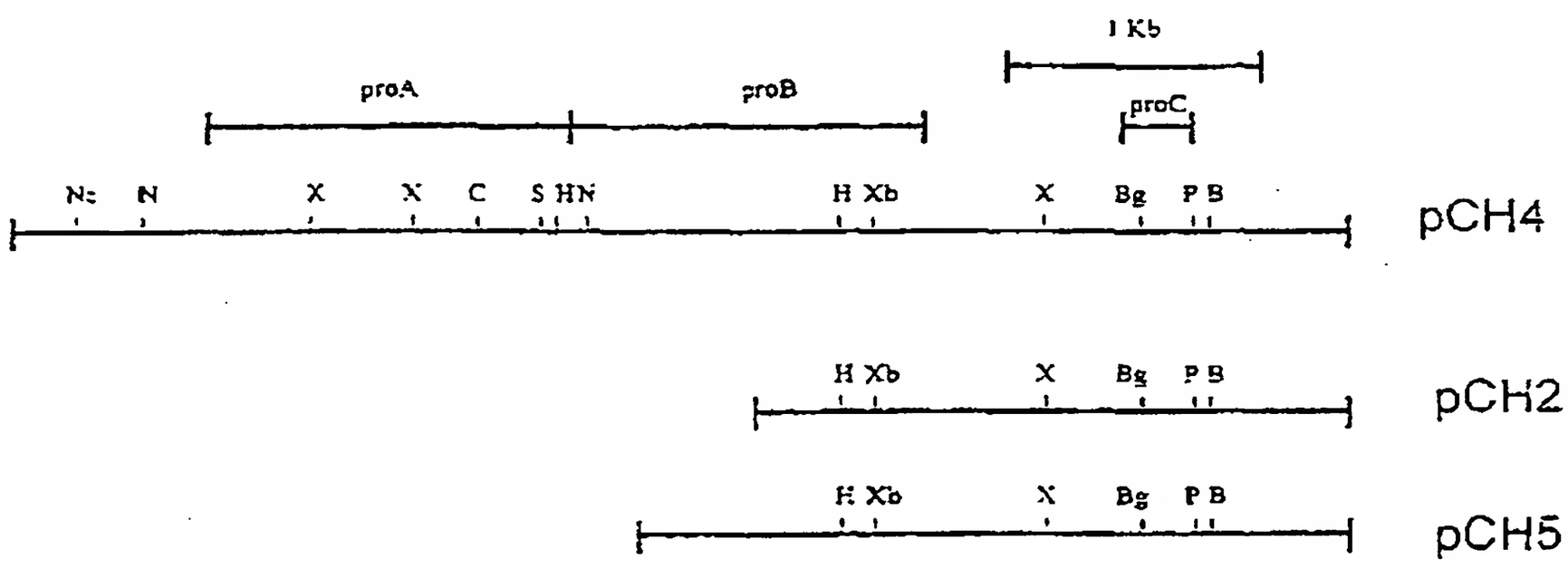
(54) Title: EHRLICHIA CANIS GENES AND VACCINES



(57) Abstract: This invention provides the sequence of 5,300 nucleotides from the E. canis genome. There are four proteins, ProA, ProB, ORF, and a cytochrome oxidase homolog, as well as a partial lipoprotein signal peptidase homolog at the carboxy terminus, coded for in this cloned fragment. The antigenic properties of these proteins allow them to be used to create a vaccine. An embodiment of this invention includes the creation of a DNA vaccine, a recombinant vaccine, and a T cell epitope vaccine.

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Ehrlichia canis Genes and Vaccines

FIELD OF THE INVENTION

The invention pertains to the field of veterinary pathogens. More particularly, the present invention pertains to the sequence of specific genes of the bacterial canine pathogen *Ehrlichia canis* and the application of this technology to the development of a vaccine.

BACKGROUND OF THE INVENTION

The present invention relates to the sequence of genes from the *E. canis* bacterium, and the development of a vaccine against this organism.

Ehrlichia canis (*E. canis*) is a small gram-negative, obligately intracellular bacterium. This bacteria is the agent which causes canine monocytic ehrlichiosis (CME), a tick-borne disease which predominantly affects dogs. The most common carrier of *E. canis* is the brown dog tick *Rhipicephalus sanguineus*. The disease was described originally in Algeria in 1935. It was subsequently recognized in the United States in 1962, but is now known throughout much of the world. Canine monocytic ehrlichiosis caused much concern during the Vietnam War, when 160 military dogs died from the *E. canis* infection. There is no vaccination currently available against *E. canis*. It is a life threatening disease that continues to be an important health concern for veterinarians and pet owners alike.

Canine monocytic ehrlichiosis is an infectious blood disease. A reduction in cellular blood elements is the primary characteristic of the disease. *E. canis* lives and reproduces in the white blood cells (leukocytes). It eventually affects the entire lymphatic system, and devastates multiple organs. By targeting the white blood cells, these cells die

off rapidly. These dead blood cells migrate primarily to the spleen, which enlarges as a result. The bone marrow recognizes the loss of the white blood cells and works to form new, healthy cells. It sends out the cells prematurely, and these immature cells do not work properly. Often, these immature cells mimic those in leukemic patients, so the disease is misdiagnosed as leukemia. Canine monocytic ehrlichiosis may predispose dogs to various cancers.

There are three stages of canine monocytic ehrlichiosis. The first, acute stage mimics a mild viral infection. During the acute stage, most, if not all, of the damage is reversible and the animal is likely to recover. This is the stage where treatment is the most effective, stressing the need for early detection. Without treatment, however, the animal will progress into a subclinical (second) stage and/or to the chronic (final) stage. When the animal has reached the chronic stage, the bacterial organism has settled within the bone marrow. Many dogs in this stage suffer massive internal hemorrhage, or develop lethal complications such as sudden stroke, heart attack, renal failure, splenic rupture or liver failure.

E. canis can be cultured *in vitro* in a mammalian-derived cell line (DH82). Continued maintenance of these cells is difficult because the cell culture must be supplemented with primary monocytes (white blood cells found in bone marrow) every two weeks. The cultures are very slow growing, and the culture media is expensive.

Data concerning the genes in the *E. canis* genome has concentrated primarily on the 16S rRNA gene. Previous work has sequenced this gene, which is a ubiquitous component of the members of the ehrlichia family, as well as the majority of organisms worldwide. The high sequence homology between this gene throughout the living world makes it a poor candidate for vaccine development. It is necessary to find other genes within this genome if hope for a vaccine against this deadly disease can ever be realized.

Sequencing of the 16S rRNA gene indicates that *E. canis* is closely related (98.2% homology) to *E. chaffeensis*, the novel etiologic agent of human ehrlichiosis. Western blots of *E. canis* are similar when probed with antisera to *E. canis*, *E. chaffeensis* and *E.*

ewingi (another cause of human ehrlichiosis) indicating a close antigenic relationship between these three species (Chen *et al.*, 1994).

The indirect fluorescent antibody test (IFA) has been developed for detecting canine monocytic ehrlichiosis. IFA detects the presence of antibodies against the invading organism in a dog's blood. Unfortunately, this test is not always accurate. Sometimes, dogs will test negative in the acute phase because their immune system is delayed in forming antibodies. Another false negative may occur if there is a low titer in the chronic stage. An additional drawback of this test is the cross-reactivity found. The anti *E. canis* polyclonal antibody positively reacts with *E. chaffeensis*, undermining the specificity of the test. An alternative test, the Giesma smear, has been used to locate the actual organism in a dog's blood. Unfortunately, despite appropriate staining techniques and intensive film examination, the organisms frequently can not be located. The fallibility of these tests makes it essential to provide better diagnostic tools for this disease.

Due to difficulties in the detection of a tick bite, early diagnosis of infection, the suppression of host defenses and the nature of persistent infection of the disease, an effective vaccine against *E. canis* is urgently needed for dogs.

SUMMARY OF THE INVENTION

This invention discloses novel sequence data for *E. canis* genes. Specifically, a clone has been identified and sequenced. Four proteins termed ProA, ProB, ORF (an open reading frame with unknown function) and a cytochrome oxidase homolog, have been identified within this clone. In addition, a partial gene encoding a lipoprotein signal peptidase homolog has been discovered.

An embodiment of this invention includes the creation of a vaccine with this sequence and protein information. The proteins disclosed in this invention are extremely antigenic. Therefore, they have the potential to be extremely useful as a vaccine. The

types of vaccine made available by this novel technology include a DNA vaccine, a recombinant vaccine, and a T cell epitope vaccine.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 shows the three clones identified in the library screen.

DESCRIPTION OF THE PREFERRED EMBODIMENT

E. canis causes a devastating canine disease. Currently, there is no vaccine available to prevent this disease. This invention provides the tools necessary to develop such a vaccine. More specifically, four genes have been identified from a genomic fragment of *E. canis*, named ProA, ProB, ORF and a cytochrome oxidase homolog. In addition, a partial gene coding for a lipoprotein signal peptidase homolog has been found. Any of these proteins can be utilized in an embodiment of this invention to develop a vaccine.

Screening an *E. canis* library

To identify genes in the *E. canis* genome, a genomic DNA expression library was constructed. An *E. canis* strain isolated from dogs with canine ehrlichiosis was grown in the dog cell line DH82 by a technique being known in the art, and incorporated by reference (Dawson *et al.*, 1991; Rikihisa, 1992). The cells were harvested and the chromosomal DNA extracted as described by a technique known in the art (Chang *et al.*, 1987; Chang *et al.*, 1989a; Chang *et al.*, 1989b; Chang *et al.*, 1993a; Chang *et al.*, 1993b). To construct the library, 200 μg of DNA was partially digested with *Sau*3A. DNA fragments from 3 to 8 kb were isolated and ligated to a plasmid, pHG165 (Stewart *et al.*, 1986). The plasmids were transformed into *E. coli* TB1 (Chang *et al.*, 1987).

The library was screened with polyclonal antibodies against *E. canis*. Polyclonal antibodies were generated from dogs that had been bitten by a tick harboring *E. canis*.

The polyclonal antibodies were preabsorbed with the lysate of an *E. coli* host strain. The library was plated on petri plates at a density of 1,000 colony forming units. Colonies were transferred to nitrocellulose and each filter was probed with 1 ml of the preabsorbed polyclonal antibodies. Positive colonies were identified with a second antibody consisting of an alkaline phosphatase-conjugated goat anti-rabbit IgG (Kirkegaard and Perry Laboratories, Gaithersburg, MD), followed by color development with a substrate solution containing nitroblue tetrazolium (NBT) and 5-bromo-4-chloro-3-indolyl phosphate (BCIP). Positive clones were rescreened three times.

Three clones were isolated from this screening procedure (Figure 1). The longest genomic fragment (pCH4) encodes four complete genes and one partial gene. It completely encodes the proteins ProA, ProB, ORF and a cytochrome oxidase homolog, as well as containing the partial sequence of a lipoprotein signal peptidase homolog. ProA and ProB are located on a single operon. Restriction endonuclease digestion mapping and DNA sequencing were done by techniques known in the art, and incorporated by reference (Chang *et. al.*, 1987; Chang *et. al.*, 1989a; Chang *et. al.*, 1989b; Chang *et. al.*, 1993a; Chang *et. al.*, 1993b). Briefly, the DNA sequence was determined by automated DNA sequencing on the ABI PRISM Model 377 DNA system. The complete nucleotide sequences were determined on both strands by primer walking. The thermal cycling of the sequencing reactions utilized the Taq DyeDeoxy™ Terminator Cycle sequencing kit. Databases were searched for homologous proteins through the use of the BLAST network service of the National Center for Biotechnology Information (NCBI) (Althchul *et al.*, 1990; Gish *et al.*, 1993).

Sequence Information

The *E. canis* genes were sequenced. The cloned fragment contains 5,300 nucleotides, and codes for four proteins. There is also one partial gene at the carboxy terminus. SEQ. ID. NO. 1 is the entire nucleotide sequence. SEQ. ID. NO. 2 and 3 are the translation of nucleotides 12 through 533 from SEQ. ID. NO. 1 and code for a cytochrome oxidase homolog. Cytochrome oxidase is important in virulence, and therefore is a strong candidate for use in a vaccine. SEQ. ID. NO. 4 and 5 are the translation of nucleotides 939 through 2,252 from SEQ. ID. NO. 1 and code for ProA. SEQ. ID. NO. 6 and 7 are the

translation of nucleotides 2,258 through 3,664 from SEQ. ID. NO. 1 and code for ProB. Preliminary evidence indicates that ProA and ProB are proteases. SEQ. ID. NO. 8 and 9 are the translation of nucleotides 4,121 through 4,795 from SEQ. ID. NO. 1 and code for ORF, a protein with unknown function. SEQ. ID. NO. 10 and 11 are the translation of the complementary sequence of nucleotides 4,884 through 5,300 from SEQ. ID. NO. 1 and code for the partial sequence of a lipoprotein signal peptidase homolog. Lipoprotein signal peptidases are membrane proteins, and by nature may be less desirable for vaccine development. However, this protein is still worth pursuing in the creation of a vaccine.

Overexpression of ProA, ProB, ORF, cytochrome oxidase and the lipoprotein signal peptidase homolog

The *E. canis* antigens are overexpressed in a T7 promoter plasmid. The pRSET vector allows high level expression in *E. coli* in the presence of T7 RNA polymerase, which has a strong affinity for the T7 promoter. After subcloning the antigen genes into the pRSET vector, the subclones are transformed into an F' *E. coli* JM109 strain. For maximum protein expression, the transformants are cultured to O.D. 600=0.3, exposed to IPTG (1 mM) for one hour and then transfected with M13/T7 bacteriophages at a multiplicity of infection (MOI) of 5-10 plaque forming units (pfu) per cell. Time course studies indicate that maximum induction is reached two hours after induction.

The pellet is harvested by centrifugation and the cells are resuspended in 6M Guanidinium (pH 7.8). Cells are ruptured by French press and the total lysate is spun at 6000 rpm to separate cell debris by a technique known in the art, and hereby incorporated by reference (Chang *et al.*, 1993c). Immobilized metal ion affinity chromatography (IMIAC) is used to purify each of the proteins under denaturing conditions as described by the manufacturer (Invitrogen, San Diego, CA). The protein samples are separated by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and visualized after staining with coomassie blue.

Vaccine Development

Prior to the present invention, no vaccine against *E. canis* had been developed. *E. canis* is endemic in dogs and closely related canidae in many parts of the world. Dogs in

North America are also increasingly at risk and the application of the present invention can potentially save the lives of thousands of dogs each year. An *E. canis* vaccine that can elicit cell-mediated immunity against this tick-borne disease of dogs is desperately needed.

DNA Vaccine

A DNA vaccine is constructed by subcloning the gene of interest into a eukaryotic plasmid vector. Candidate vectors include, but are not limited to, pcDNA3, pCI, VR1012, and VR1020. This construct is used as a vaccine.

Each of the newly identified genes, ProA, ProB, ORF, the cytochrome oxidase homolog, or the partial lipoprotein signal peptidase homolog can be used to create a DNA vaccine (reviewed in Robinson, 1997). In addition, any immunologically active portion of these proteins is a potential candidate for the vaccine. A plasmid containing one of these genes in an expression vector is constructed. The gene must be inserted in the correct orientation in order for the genes to be expressed under the control of eukaryotic promoters. Possible promoters include, but are not limited to, the cytomegalovirus (CMV) immediate early promoter, the human tissue plasminogen activator (t-PA) gene (characterized in Degen *et al.*, 1986), and the promoter/enhancer region of the human elongation factor alpha (EF-1 α) (characterized in Uetsuki *et al.*, 1989). Orientation is identified by restriction endonuclease digestion and DNA sequencing.

Expression of these gene products is confirmed by indirect immunofluorescent staining of transiently transfected COS cells. The same plasmid without these genes is used as a control. Plasmid DNA is transformed into *Escherichia coli* DH5α. DNA is purified by cesium chloride gradients and the concentration is determined by a standard protocol being known in the art, and incorporated by reference (Nyika *et al.*, 1998).

Once the vector is purified, the vector containing the DNA can be suspended in phosphate buffer saline solution and directly injected into dogs. Inoculation can be done via the muscle with a needle or intraveneously. Alternatively, a gene gun can be used to transport DNA-coated gold beads into cells by a technique known in the art, and hereby incorporated by reference (Fynan *et al.*, 1993). The rationale behind this type of vaccine

is that the inoculated host expresses the plasmid DNA in its cells, and produces a protein that raises an immune response. Each of the newly identified genes can be used to create a vaccine by this technique.

CpG molecules can be used as an adjuvant in the vaccine. This technique is known in the art, and is hereby incorporated by reference (Klinman *et al.*, 1997). Adjuvants are materials that help antigens or increase the immune response to an antigen. The motifs consist of an unmethylated CpG dinucleotide flanked by two 5' purines and two 3' pyrimidines. Oligonucleotides containing CpG motifs have been shown to activate the immune system, thereby boosting an antigen-specific immune response. This effect can be utilized in this invention by mixing the CpG oligonucleotides with the DNA vaccine, or physically linking the CpG motifs to the plasmid DNA.

Recombinant Vaccine

In order to develop a recombinant vaccine, each of the genes is individually subcloned into overexpression vectors, and then purified for vaccine development. ProA, ProB, ORF, the cytochrome oxidase homolog or the partial lipoprotein signal peptidase homolog is expressed in a plasmid with a strong promoter such as the tac, T5, or T7 promoter. Alternatively, immunologically active fragments of these proteins are used in the development of a vaccine. Each of these genes is subcloned into a plasmid and transformed into an *E. coli* strain as described above.

The recombinant protein is overexpressed using a vector with a strong promoter. Vectors for use in this technique include pREST (Invitrogen Inc., CA), pKK233-3 (Pharmacia, CA), and the pET system (Promega, WI), although any vector with a strong promoter can be used. After overexpression, the proteins are purified and mixed with adjuvant. Potential adjuvants include, but are not limited to, aluminum hydroxide, QuilA, or Montamide. The purified protein is used as immunogen to vaccinate dogs by a technique being known in the art, and incorporated by reference (Chang *et al.*, 1993c; Chang *et al.*, 1995). Briefly, the individual protein is expressed and purified from *E. coli*. Then, the dogs are injected intramuscularly or subcutaneously with the purified recombinant vaccine and adjuvant. This injection elicits an immune response.

T Cell Epitope Vaccine

Direct cell cytoxicity mediated by $CD8^+$ T lymphocytes (CTL) is the major mechanism of defense against intracellular pathogens. These effector lymphocytes eliminate infected cells by recognizing short peptides associated with MHC class I molecules on the cell surface. Exogenous antigens enter the endosomal pathway and are presented to $CD4^+$ T cells in association with class II molecules whereas endogenously synthesized antigens are presented to $CD8^+$ T cells in association with MHC class I molecules. *E. canis* is an intracellular pathogen that resides in monocytes and macrophages. The present invention develops novel ways of generating an *E. canis*-specific CTL response that would eliminate the organism from monocytes or macrophages of infected animals.

A strategy for increasing the protective response of a protein vaccine is to immunize with selective epitopes of the protein. The rationale behind this is that an epitope vaccine contains the most relevant immunogenic peptide components without the irrelevant portions. Therefore, a search is performed for the most highly antigenic portions of the newly identified proteins.

To identify T-cell epitopes from the newly discovered proteins, an initial electronic search for homologous sequences to known T-cell epitopes is performed. In addition, extensive T-cell epitope mapping is carried out. Each of the proteins, ProA, ProB, ORF, the cytochrome oxidase homolog, and the partial lipoprotein signal peptidase homolog, is tested for immunogenic peptide fragments. Mapping of T cell epitopes by a technique known in the art is hereby incorporated by reference (Launois *et al.*, 1994; Lee and Horwitz, 1999). Briefly, short, overlapping peptide sequences (9-20 amino acids) are synthesized over the entire length of the protein in question. These short peptide fragments are tested using healthy dogs which have been immunized with the protein of interest. Peripheral blood mononuclear cells from the dogs are tested for T cell stimulatory and IFN-γ inducing properties. Those fragments which elicit the strongest response are the best candidates for a T-cell epitope vaccine.

Once fragments are identified which will make the best epitopes, a recombinant adenylate cyclase of *Bordetella bronchiseptica* is constructed carrying an *E. canis* $CD8^+$ T cell epitope. The adenylate cyclase toxin (CyaA) of *Bordetella bronchiseptica* causes disease in dogs and elicits an immune response. In addition, CyaA is well suited for intracytoplasmic targeting. Its catalytic domain (AC), corresponding to the N-terminal 400 amino acid residues of the 1,706-residue-long protein, can be delivered to many eukaryotic cells, including cells of the immune system. Also, toxin internalization is independent of receptor-mediated endocytosis, suggesting that the catalytic domain can be delivered directly to the cytosol of target cells through the cytoplasmic membrane. The *Pseudomonas aeruginosa* exotoxin A (PE) is another toxin which could be used in this procedure to deliver peptides or proteins into cells, by a technique known in the art, and hereby incorporated by reference (Donnelly *et al.*, 1993).

Foreign peptides (16 residues) have been inserted into various sites of the AC domain of CyaA without altering its stability or catalytic and calmodulin-binding properties. Thus, protein engineering allows the design and delivery of antigens that specifically stimulate CTLs. The induction of specific $CD8^+$ T cells can play an important role in canine ehrlichiosis control due to the intracellular persistence of *E. canis* in monocytes.

The adenylate cyclase (AC) toxin (*cya)* gene of *B. bronchiseptica* has been cloned. A synthetic double-stranded oligonucleotide encoding a 9 to 20 amino acid class I T cell epitope of either ProA, ProB, ORF, the cytochrome oxidase homolog, or the partial lipoprotein signal peptidase homolog, is designed according to *B. bronchiseptica* codon usage. The complementary oligonucleotides are inserted in the hypervariable region of the cloned AC-coding sequence of the *cya*. This technique is known in the art in other systems, and is incorporated by reference (Sebo *et al.*, 1995; Guermonprez *et al.*, 1999).

Recombinant plasmids carrying the chimeric *cya* gene are sequenced to determine the copy number and orientation of the inserted epitope. A plasmid with a complete copy of the insert that specifies the T-cell epitope ($CD8^+$) in the correct orientation is chosen from the sequenced plasmids. The ability of the new chimeric protein to enter eukaryotic cells is necessary to ensure intracellular targeting of the epitopes (Fayolle *et al.*, 1996).

A vaccine can be created in one of two ways. Recombinant chimeric protein can be purified and used to inoculate dogs. Alternatively, an attenuated *B. bronchiseptica* strain that carries a T-cell epitope or *E. canis* gene by in-frame insertion into adenylate cyclase is created by allelic-exchange. Allelic-exchange is a technique known in the art, and is hereby incorporated by reference (Cotter and Miller, 1994).

Finally, protection against *E. canis* infection in dogs vaccinated with the adenylase cyclase- ProA, ProB, ORF, cytochrome oxidase homolog, or lipoprotein signal peptidase homolog chimeric protein is determined. Wild type and recombinant ACs and CyAs are diluted to working concentrations in PBS and the chimeric protein is injected into dogs either intramuscularly or subcutaneously. Alternatively, the T-cell epitope is inserted into the adenylate cyclase gene of an attenuated *B. bronchiseptica* strain in frame, and the dogs are given the live bacteria.

Recombinant antigens are promising candidates for human and animal vaccination against various pathogens. However, a serious drawback is the poor immunogenicity of recombinant antigens as compared to native antigens. A major challenge in the development of a new recombinant vaccine is, therefore, to have a new adjuvant system that increases the immunogenicity of antigens. Cytokines are powerful immunoregulatory molecules. Cytokines which could be used as adjuvants in this invention include, but are not limited to, IL-12 (interleukin-12), GM-CSF (granulocyte-macrophage colony stimulating factor), IL-1β (interleukin-1β) and γ-IFN (gamma interferon).

These cytokines can have negative side effects including pyrogenic and/or proinflammatory symptoms in the vaccinated host. Therefore, to avoid the side effects of a whole cytokine protein, an alternate approach is to use synthetic peptide fragments with the desired immunostimulatory properties. The nonapeptide sequence VQGEESNDK of IL-1β protein is endowed with powerful immuno-enhancing properties, and is discussed here to illustrate the use of a cytokine to increase immunogenicity.

This nonapeptide is inserted into the ProA, ProB, ORF, the cytochrome oxidase homolog, or the partial lipoprotein signal peptidase homolog protein and its immunogenicity is compared to that of the native protein. Reportedly, the insertion of this

sequence into a poorly immunogenic recombinant antigen increases the chance of a strong protective immune response after vaccination. This peptide could enhance the *in vivo* immune response against both T-dependent and T-independent antigens. The canine IL-1β sequence may mimic many immunomodulatory activities of the entire molecule of IL-1β while apparently lacking many of its undesirable proinflammatory properties. This strategy is employed to increase the immunogenicity of ProA, ProB, ORF, cytochrome oxidase, the partial lipoprotein signal peptidase homolog and other *E. canis* antigens.

Plasmid pYFC199 is derived from a pBR322 plasmid by the insertion of a fragment that includes the ProA, ProB, ORF, the cytochrome oxidase homolog, or the partial lipoprotein signal peptidase protein from *E. canis*. This plasmid contains a unique *Hind*III site where in-frame insertions encoding exogenous sequences can be inserted. Two complementary oligonucleotides,
AGGCTTGTTCAGGGTGAAGAAGAATCCAACGACAAAAGCTT and
AAGCTTTTGTCGTTGGATTCTTCACCCTGAACTTGCCA, that encode the canine IL-1β 163-171 peptide are annealed, cut with *Hind*III, and inserted into the pYFC199 *Hind*III site. The recombinant plasmid carrying the chimeric IL-1β gene is sequenced to determine the orientation of the inserted epitope.

The efficacy of the recombinant proteins as vaccines is tested in dogs. The purified protein is injected intraperitoneally into dogs. Specific pathogen free (SPF) dogs are divided into five groups: one group is given recombinant adenylate cyclase of *Bordetella bronchiseptica* carrying *E. canis* $CD8^+$ T cell epitopes derived from ProA, ProB, ORF, cytochrome oxidase homolog, or the partial lipoprotein signal peptidase homolog, one group is given recombinant adenylate cyclase of *Bordetella bronchiseptica* as a control, one group is given the ProA, ProB, ORF, cytochrome oxidase homolog, or the partial lipoprotein signal peptidase homolog protein plus a canine IL-1β 163-171 insert, one group is given a T cell epitope derived from ProA, ProB, ORF, cytochrome oxidase homolog, or the partial lipoprotein signal peptidase homolog alone, and the last group is given PBS as a negative control.

All animals are vaccinated (30-40 μg each) four times. The dogs are challenged ten days after the last vaccination with 10^7 *E. canis*. At day five postchallenge, approximately 1 ml blood from each dog is collected in an EDTA tube. Whether the vaccinated groups eliminate the organisms as compared to that of the control group is tested by culture and PCR. Two primers derived from the genes cloned can be used to amplify the gene product from the tissues or blood samples from these dogs. The internal primer can also be designed for use as an oligonucleotide probe to hybridize the PCR gene product.

This invention provides a badly needed vaccine against the *E. canis* bacterium. The vaccine can be used to protect dogs throughout the world from canine monocytic ehrlichiosis.

Accordingly, it is to be understood that the embodiments of the invention herein described are merely illustrative of the application of the principles of the invention. Reference herein to details of the illustrated embodiments are not intended to limit the scope of the claims, which themselves recite those features regarded as essential to the invention.

What is claimed is:

1. A recombinant DNA comprising said DNA selected from the group consisting of:

a) a recombinant DNA that encodes a protein having an amino acid sequence as shown in SEQ. ID. NO. 3;

b) a recombinant DNA that encodes a protein having an amino acid sequence as shown in SEQ. ID. NO. 5;

c) a recombinant DNA that encodes a protein having an amino acid sequence as shown in SEQ. ID. NO. 7;

d) a recombinant DNA that encodes a protein having an amino acid sequence as shown in SEQ. ID. NO. 9;

e) a recombinant DNA that encodes a protein having an amino acid sequence as shown in SEQ. ID. NO. 11; and

f) any portion of said DNA above that encodes a protein that elicits an immune response against *E. canis*.

2. The recombinant DNA of claim 1 wherein said DNA encodes at least one immunogenic epitope.

3. A recombinant protein comprising said protein selected from the group consisting of:

a) a protein having an amino acid sequence as shown in SEQ. ID. NO. 3;

b) a protein having an amino acid sequence as shown in SEQ. ID. NO. 5;

c) a protein having an amino acid sequence as shown in SEQ. ID. NO. 7;

d) a protein having an amino acid sequence as shown in SEQ. ID. NO. 9;

e) a protein having an amino acid sequence as shown in SEQ. ID. NO. 11; and

f) any portion of any of the above proteins that elicits an immune response against *E. canis*.

4. The recombinant protein of claim 3 wherein said protein includes at least one immunogenic epitope.

5. A vaccine wherein said vaccine protects dogs against *E. canis* infection.

6. The vaccine of claim 5 comprising:

a) a vector capable of expressing a recombinant DNA inserted into said vector such that a recombinant protein is expressed when said vector is provided in an appropriate host; and

b) the recombinant DNA inserted into said vector wherein said DNA is selected from the group consisting of:

i. a recombinant DNA that encodes a protein having an amino acid sequence as shown in SEQ. ID. NO. 3;

ii. a recombinant DNA that encodes a protein having an amino acid sequence as shown in SEQ. ID. NO. 5;

iii. a recombinant DNA that encodes a protein having an amino acid sequence as shown in SEQ. ID. NO. 7;

iv. a recombinant DNA that encodes a protein having an amino acid sequence as shown in SEQ. ID. NO. 9;

v. a recombinant DNA that encodes a protein having an amino acid sequence as shown in SEQ. ID. NO. 11; and

vi. any portion of said DNA above that encodes a protein that elicits an immune response against *E. canis*.

7. The vaccine of claim 6, wherein said DNA further comprises DNA that encodes CpG motifs.

8. The vaccine of claim 6 wherein said DNA further comprises a promoter selected from the group consisting of:

a) a cytomegalovirus (CMV) immediate early promoter;

b) a human tissue plasminogen activator gene (t-PA); and

c) a promoter/enhancer region of a human elongation factor alpha (EF-1 α).

9. The vaccine of claim 6, wherein said vector is selected from the group consisting of:

a) pcDNA3;

b) pC1;

c) VR1012; and

d) VR1020.

10. The vaccine of claim 6 wherein said vaccine is administered into said host by a method selected from the group consisting of:

a) intramuscular injection;

b) intraveneous injection; and

c) gene gun injection.

11. The vaccine of claim 10, wherein said host is a dog.

12. The vaccine of claim 5 comprising:

a) a recombinant protein that is selected from the group consisting of:

i. a protein having an amino acid sequence as shown in SEQ. ID. NO. 3;

ii. a protein having an amino acid sequence as shown in SEQ. ID. NO. 5;

iii. a protein having an amino acid sequence as shown in SEQ. ID. NO. 7;

iv. a protein having an amino acid sequence as shown in SEQ. ID. NO. 9;

v. a protein having an amino acid sequence as shown in SEQ. ID. NO. 11; and

vi. any portion of any of the above proteins that elicits an immune response against *E. canis*.

13. The vaccine of claim 12, wherein said vaccine further comprises adjuvants selected from the group consisting of:

a) aluminum hydroxide;

b) QuilA; and

c) Montamide.

14. The vaccine of claim 12 further comprising a cytokine operatively associated with said recombinant protein.

15. The vaccine of claim 14 wherein said cytokine is selected from the group consisting of:

a) interleukin-1β (IL-1β);

b) granulocyte-macrophage colony stimulating factor (GM-CSF);

c) gamma interferon (γ-IFN);

d) amino acids VQGEESNDK from the IL-Iβ protein: and

e) any portion of any of the cytokines above that elicits an improved immunogenic response against *E. canis*.

16. The vaccine of claim 12 wherein said vaccine is administered into a host by a method selected from the group consisting of:

a) intramuscular injection; and

b) subcutaneous injection.

17. The vaccine of claim 16 wherein said host is a dog.

18. The vaccine of claim 5 comprising a recombinant protein that includes a T cell epitope wherein said T cell epitope comprises an amino acid peptide fragment of a protein selected from the group consisting of:

a) a protein having an amino acid sequence as shown in SEQ. ID. NO. 3;

b) a protein having an amino acid sequence as shown in SEQ. ID. NO. 5;

c) a protein having an amino acid sequence as shown in SEQ. ID. NO. 7;

d) a protein having an amino acid sequence as shown in SEQ. ID. NO. 9;

e) a protein having an amino acid sequence as shown in SEQ. ID. NO. 11; and

f) any portion of any of the above proteins that elicits an immune response against *E. canis*.

19. The vaccine of claim 18 wherein said amino acid peptide fragment comprises nine to twenty amino acids.

20. The vaccine of claim 18 further comprising a recombinant DNA encoding a protein which is capable of being internalized into eukaryotic cells, including cells of the immune system.

21. The vaccine of claim 20 wherein said protein capable of being internalized into eukaryotic cells comprises a toxin selected from the group consisting of:

a) a recombinant adenylate cyclase of *Bordetella bronchiseptica*; and

b) a recombinant exotoxin A (PE) of *Pseudomonas aeruginosa*.

22. The vaccine of claim 18 wherein said vaccine is administered into a host by a method selected from the group consisting of:

a) intramuscular injection; and

b) subcutaneous injection.

23. The vaccine of claim 22 wherein said host is a dog.

24. A method of identifying a T cell epitope against *E. canis* comprising:

a) synthesizing overlapping peptide fragments over an entire length of a protein wherein said protein is selected from the group consisting of:

i. a protein having an amino acid sequence as shown in SEQ. ID. NO. 3;

ii. a protein having an amino acid sequence as shown in SEQ. ID. NO. 5;

iii. a protein having an amino acid sequence as shown in SEQ. ID. NO. 7;

iv. a protein having an amino acid sequence as shown in SEQ. ID. NO. 9;

v. a protein having an amino acid sequence as shown in SEQ. ID. NO. 11; and

vi. any portion of any of the proteins above that elicits an immune response *against E. canis*;

b) testing said peptide fragment to determine if said peptide fragment elicits an immune response in a host animal; and

c) identifying said peptide fragment as said T cell epitope of *E. canis* if said fragment elicits an immune response.

25. The method of claim 24 wherein said peptide fragment comprises nine to twenty amino acids.

26. A method of creating a vaccine against *E. canis* comprising:

a) selecting a vector capable of expressing a recombinant DNA inserted into said vector; and

b) inserting a recombinant DNA into said vector such that a recombinant protein is expressed when said vector is provided in an appropriate host wherein said DNA is selected from the group consisting of:

i. a recombinant DNA that encodes a protein having an amino acid sequence as shown in SEQ. ID. NO. 3;

ii. a recombinant DNA that encodes a protein having an amino acid sequence as shown in SEQ. ID. NO. 5;

iii. a recombinant DNA that encodes a protein having an amino acid sequence as shown in SEQ. ID. NO. 7;

iv. a recombinant DNA that encodes a protein having an amino acid sequence as shown in SEQ. ID. NO. 9;

v. a recombinant DNA that encodes a protein having an amino acid sequence as shown in SEQ. ID. NO. 11; and

vi. any portion of said DNA above that encodes a protein that elicits an immune response against *E. canis*.

27. The method of claim 26, wherein said DNA further comprises DNA that encodes CpG motifs.

28. The method of claim 26 wherein said DNA further comprises a promoter selected from the group consisting of:

a) a cytomegalovirus (CMV) immediate early promoter;

b) a human tissue plasminogen activator gene (t-PA); and

c) a promoter/enhancer region of a human elongation factor alpha (EF-1 α).

29. The method of claim 26, wherein said vector is selected from the group consisting of:

a) pcDNA3;

b) pC1;

c) VR1012; and

d) VR1020.

30. The method of claim 26 wherein said vaccine is injected into said host in a manner selected from the group consisting of:

a) intramuscular injection;

b) intraveneous injection; and

c) gene gun injection.

31. The method of claim 30, wherein said host is a dog.

32. A method of creating a vaccine against *E. canis* comprising:

a) selecting a vector capable of expressing a recombinant protein inserted into said vector;

b) insertion of a recombinant DNA into said vector such that said recombinant protein is expressed when said vector is transformed into a bacterial strain wherein said DNA is selected from the group consisting of:

i. a recombinant DNA that encodes a protein having an amino acid sequence as shown in SEQ. ID. NO. 3;

ii. a recombinant DNA that encodes a protein having an amino acid sequence as shown in SEQ. ID. NO. 5;

iii. a recombinant DNA that encodes a protein having an amino acid sequence as shown in SEQ. ID. NO. 7;

iv. a recombinant DNA that encodes a protein having an amino acid sequence as shown in SEQ. ID. NO. 9;

v. a recombinant DNA that encodes a protein having an amino acid sequence as shown in SEQ. ID. NO. 11; and

vi. any portion of said DNA above that encodes a protein that elicits an immune response against *E. canis*; and

c) harvesting said recombinant protein from said bacterial strain.

33. The method of claim 32, wherein said vaccine further comprises adjuvants selected from the group consisting of:

a) aluminum hydroxide;

b) QuilA; and

c) Montamide.

34. The method of claim 32, wherein said vaccine further comprises a promoter selected from the group consisting of:

a) tac;

b) T5; and

c) T7.

35. The method of claim 32, wherein said bacterial strain is *E. coli*.

36. The method of claim 32, wherein said vector is selected from the group consisting of:

a) pREST;

b) pET; and

c) pKK233-3.

37. The method of claim 32 wherein said vaccine further comprises a cytokine operatively associated with said vaccine.

38. The method of claim 37 wherein said cytokine is selected from the group consisting of:

a) interleukin-1β (IL-1β);

b) granulocyte-macrophage colony stimulating factor (GM-CSF);

c) gamma interferon (γ-IFN);

d) amino acids VQGEESNDK from the IL-Iβ protein; and

e) any portion of any of the cytokines above that elicits an improved immunogenic response against *E. canis*.

39. The method of claim 32 wherein said vaccine is injected into said host in a manner selected from the group consisting of:

a) intramuscular injection; and

b) subcutaneous injection.

40. The method of claim 39 wherein said host is a dog.

41. A method of creating a T cell epitope vaccine comprising:

a) selecting a recombinant protein that includes a T cell epitope wherein said T cell epitope comprises an amino acid peptide fragment of a protein selected from the group consisting of:

i. a protein having an amino acid sequence as shown in SEQ. ID. NO. 3;

ii. a protein having an amino acid sequence as shown in SEQ. ID. NO. 5;

iii. a protein having an amino acid sequence as shown in SEQ. ID. NO. 7;

iv. a protein having an amino acid sequence as shown in SEQ. ID. NO. 9;

v. a protein having an amino acid sequence as shown in SEQ. ID. NO. 11; and

vi. any portion of any of the above proteins that elicits an immune response against *E. canis*;

b) identifying said T cell epitope from said protein;

c) incorporating said T cell epitope into a construct capable of expressing said epitope as a protein; and

d) harvesting said protein.

42. The method of claim 41 wherein said amino acid peptide fragment comprises nine to twenty amino acids.

43. The method of claim 41 wherein said construct capable of expressing said epitope further comprises a recombinant DNA encoding a protein which is capable of being internalized into eukaryotic cells, including cells of the immune system.

44. The method of claim 43 wherein said protein capable of being internalized into eukaryotic cells comprises a toxin selected from the group consisting of:

a) a recombinant adenylate cyclase of *Bordetella bronchiseptica*; and

b) a recombinant exotoxin A (PE) of *Pseudomonas aeruginosa*.

45. The method of claim 41 wherein said vaccine is injected into said host in a manner selected from the group consisting of:

a) intramuscular injection; and

4 b) subcutaneous injection.

1 46. The method of claim 45 wherein said host is a dog.

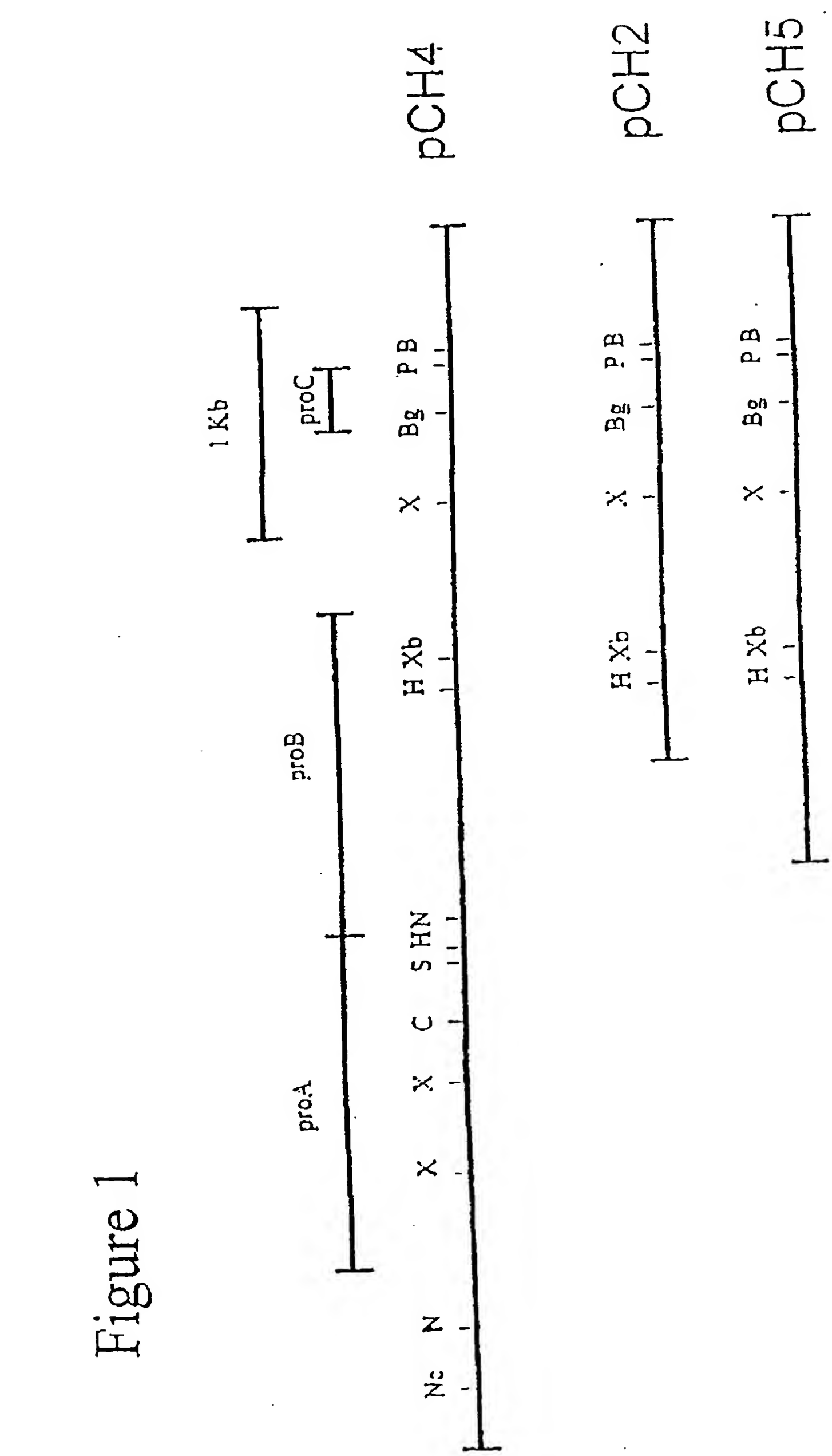

Figure 1

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Leu Glu Asn Gly Ile Ser Ala Glu Tyr Leu Glu Ser Ala Lys Tyr Lys
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gta aaa gca cat tta act tat gca ttt gac gga cta act ttc ata tca  1152
Val Lys Ala His Leu Thr Tyr Ala Phe Asp Gly Leu Thr Phe Ile Ser
    370                 375                 380

tat ttt tat ggc atg cat cta ata cta gga gta ccg cta tca gaa atc  1200
Tyr Phe Tyr Gly Met His Leu Ile Leu Gly Val Pro Leu Ser Glu Ile
385                 390                 395                 400

agt aat att tac gat acc ata gac aaa gta agt atc caa gat gtt aac  1248
Ser Asn Ile Tyr Asp Thr Ile Asp Lys Val Ser Ile Gln Asp Val Asn
                405                 410                 415

tcc gct atg gaa aat atc ttt caa aac aat ata aga tta acc ggg cat  1296
Ser Ala Met Glu Asn Ile Phe Gln Asn Asn Ile Arg Leu Thr Gly His
            420                 425                 430

tta tta cct aat gga gaa                                         1314
Leu Leu Pro Asn Gly Glu
        435
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<210> 5

<211> 438

<212> PRT

<213> Ehrlichia canis

<400> 5

Met Met Lys Phe Phe Thr Cys Phe Phe Ile Val Phe Leu Thr Ile Ala
1 5 10 15

Asn His Ala Leu Ser Phe Asn Ile Lys Val Thr His Glu Lys Leu Asp
20 25 30

Asn Gly Met Glu Val Tyr Val Ile Pro Asn His Arg Ala Pro Ala Val
35 40 45

Met His Met Val Leu Tyr Lys Val Gly Gly Thr Asp Asp Pro Val Gly
50 55 60

Tyr Ser Gly Leu Ala His Phe Phe Glu His Leu Met Phe Ser Gly Thr
65 70 75 80

Glu Lys Phe Pro Asn Leu Ile Ser Thr Leu Ser Asn Ile Gly Gly Asn
85 90 95

Phe Asn Ala Ser Thr Ser Gln Phe Cys Thr Ile Tyr Tyr Glu Leu Ile
100 105 110

Pro Lys Gln Tyr Leu Ser Leu Ala Met Asp Ile Glu Ser Asp Arg Met
115 120 125

Gln Asn Phe Lys Val Thr Asp Lys Ala Leu Ile Arg Glu Gln Lys Val
130 135 140

Val Leu Glu Glu Arg Lys Met Arg Val Glu Ser Gln Ala Lys Asn Ile
145 150 155 160

Leu Glu Glu Glu Met Glu Asn Ala Phe Tyr Tyr Asn Gly Tyr Gly Arg
165 170 175

Pro Val Val Gly Trp Glu His Glu Ile Ser Asn Tyr Asn Lys Glu Val
180 185 190

Ala Glu Ala Phe His Lys Leu His Tyr Ser Pro Asn Asn Ala Ile Leu
195 200 205

Ile Val Thr Gly Asp Ala Asp Pro Gln Glu Val Ile Thr Leu Ala Lys
210 215 220

Gln Tyr Tyr Gly Lys Ile Pro Ser Asn Asn Lys Lys Pro Ser Ser Gln
225 230 235 240

Val Arg Val Glu Pro Pro His Lys Thr Asn Met Thr Leu Thr Leu Lys
245 250 255

Asp Ser Ser Val Glu Ile Pro Glu Leu Phe Leu Met Tyr Gln Ile Pro
260 265 270

Asn Gly Ile Thr Asn Lys Asn Tyr Ile Leu Asn Met Met Leu Ala Glu
275 280 285

Ile Leu Gly Ser Gly Lys Phe Ser Leu Leu Tyr Asn Asp Leu Val Ile
290 295 300

Asn Asn Pro Ile Val Thr Ser Ile Lys Thr Asp Tyr Asn Tyr Leu Thr
305 310 315 320

Asp Ser Asp Asn Tyr Leu Ser Ile Glu Ala Ile Pro Lys Asn Gly Ile
325 330 335

Ser Thr Glu Ala Val Glu Gln Glu Ile His Lys Cys Ile Asn Asn Tyr
340 345 350

Leu Glu Asn Gly Ile Ser Ala Glu Tyr Leu Glu Ser Ala Lys Tyr Lys
355 360 365

Val Lys Ala His Leu Thr Tyr Ala Phe Asp Gly Leu Thr Phe Ile Ser
370 375 380

Tyr Phe Tyr Gly Met His Leu Ile Leu Gly Val Pro Leu Ser Glu Ile
385 390 395 400

Ser Asn Ile Tyr Asp Thr Ile Asp Lys Val Ser Ile Gln Asp Val Asn
405 410 415

Ser Ala Met Glu Asn Ile Phe Gln Asn Asn Ile Arg Leu Thr Gly His
420 425 430

Leu Leu Pro Asn Gly Glu
435

<210> 6

<211> 1407

<212> DNA

<213> Ehrlichia canis

<220>

<221> CDS

<222> (1)..(1407)

<223> Protein translated from 2,258 through 3,664
(ProB).

<400> 6

atg aga aac ata ttg tgt tac aca tta ata ttg att ttc ttt tca ttc 48
Met Arg Asn Ile Leu Cys Tyr Thr Leu Ile Leu Ile Phe Phe Ser Phe
1 5 10 15

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aat aca tat gca aat gat ctc aat att aac ata aaa gaa gct aca act    96
Asn Thr Tyr Ala Asn Asp Leu Asn Ile Asn Ile Lys Glu Ala Thr Thr
            20                  25                  30

aaa aat aaa ata cac tat cta tat gtt gaa cat cat aac cta cca aca   144
Lys Asn Lys Ile His Tyr Leu Tyr Val Glu His His Asn Leu Pro Thr
        35                  40                  45

att tcc tta aaa ttt gca ttc aag aaa gca gga tac gct tat gat gcc   192
Ile Ser Leu Lys Phe Ala Phe Lys Lys Ala Gly Tyr Ala Tyr Asp Ala
    50                  55                  60

ttt gat aag caa gga ctt gca tac ttt aca tca aaa ata tta aac gaa   240
Phe Asp Lys Gln Gly Leu Ala Tyr Phe Thr Ser Lys Ile Leu Asn Glu
65                  70                  75                  80

gga tca aaa aac aac tat gct ctc agt ttt gca caa caa tta gaa ggc   288
Gly Ser Lys Asn Asn Tyr Ala Leu Ser Phe Ala Gln Gln Leu Glu Gly
                85                  90                  95

aaa ggt ata gac tta aaa ttt gat ata gac cta gac aat ttt tat ata   336
Lys Gly Ile Asp Leu Lys Phe Asp Ile Asp Leu Asp Asn Phe Tyr Ile
            100                 105                 110

tca tta aaa acc tta tca gaa aac ttt gaa gaa gcc cta gtt tta ctc   384
Ser Leu Lys Thr Leu Ser Glu Asn Phe Glu Glu Ala Leu Val Leu Leu
        115                 120                 125

agt gat tgc ata ttc aac acc gtc aca gat caa gaa ata ttc aat aga   432
Ser Asp Cys Ile Phe Asn Thr Val Thr Asp Gln Glu Ile Phe Asn Arg
    130                 135                 140

ata ata gca gaa cag att gca cat gtt aaa tca tta tat tct gct cct   480
Ile Ile Ala Glu Gln Ile Ala His Val Lys Ser Leu Tyr Ser Ala Pro
145                 150                 155                 160

gaa ttt ata gct aca aca gaa atg aat cac gct ata ttc aaa ggg cac   528
Glu Phe Ile Ala Thr Thr Glu Met Asn His Ala Ile Phe Lys Gly His
                165                 170                 175

cca tat tct aac aaa gtt tac ggg aca tta aat aca atc aat aat atc   576
Pro Tyr Ser Asn Lys Val Tyr Gly Thr Leu Asn Thr Ile Asn Asn Ile
            180                 185                 190

aac cag gaa gac gtt gca tta tat ata aaa aat agt ttt gac aag gaa   624
Asn Gln Glu Asp Val Ala Leu Tyr Ile Lys Asn Ser Phe Asp Lys Glu
        195                 200                 205

caa atc gtt atc agc gca gca gga gat gta gat cca aca cag cta tca   672
Gln Ile Val Ile Ser Ala Ala Gly Asp Val Asp Pro Thr Gln Leu Ser
    210                 215                 220

aat tta cta gat aaa tat att ctt tcc aaa ttg cca tct ggt aat aac   720
Asn Leu Leu Asp Lys Tyr Ile Leu Ser Lys Leu Pro Ser Gly Asn Asn
225                 230                 235                 240
```

```
aaa aat acc ata cca gat acg act gtt aat aga gaa gac aca tta tta   768
Lys Asn Thr Ile Pro Asp Thr Thr Val Asn Arg Glu Asp Thr Leu Leu
                245                 250                 255

tat gta cag aga gat gta cca caa agt gtc ata atg ttt gct aca gac   816
Tyr Val Gln Arg Asp Val Pro Gln Ser Val Ile Met Phe Ala Thr Asp
            260                 265                 270

aca gta cca tat cac agc aaa gac tat cat gca tca aac ttg ttc aat   864
Thr Val Pro Tyr His Ser Lys Asp Tyr His Ala Ser Asn Leu Phe Asn
        275                 280                 285

act atg cta ggc gga tta agt ctc aat tca ata tta atg ata gaa tta   912
Thr Met Leu Gly Gly Leu Ser Leu Asn Ser Ile Leu Met Ile Glu Leu
    290                 295                 300

aga gac aag tta gga tta aca tac cat agt agc agt tca cta tct aac   960
Arg Asp Lys Leu Gly Leu Thr Tyr His Ser Ser Ser Ser Leu Ser Asn
305                 310                 315                 320

atg aat cat agt aat gtg cta ttt ggt aca ata ttc act gat aat acc  1008
Met Asn His Ser Asn Val Leu Phe Gly Thr Ile Phe Thr Asp Asn Thr
                325                 330                 335

aca gta aca aaa tgt ata tcc gtc tta aca gat att ata gag cac att  1056
Thr Val Thr Lys Cys Ile Ser Val Leu Thr Asp Ile Ile Glu His Ile
            340                 345                 350

aaa aag tat gga gtt gat gaa gac act ttt gca att gca aaa tct agt  1104
Lys Lys Tyr Gly Val Asp Glu Asp Thr Phe Ala Ile Ala Lys Ser Ser
        355                 360                 365

att acc aac tct ttt att tta tct atg tta aat aac aat aat gtt agt  1152
Ile Thr Asn Ser Phe Ile Leu Ser Met Leu Asn Asn Asn Asn Val Ser
    370                 375                 380

gag ata ttg tta agc tta caa tta cac gat cta gat ccg agt tat att  1200
Glu Ile Leu Leu Ser Leu Gln Leu His Asp Leu Asp Pro Ser Tyr Ile
385                 390                 395                 400

aat aaa tac aat tct tac tac aaa gca ata aca ata gaa gaa gta aat  1248
Asn Lys Tyr Asn Ser Tyr Tyr Lys Ala Ile Thr Ile Glu Glu Val Asn
                405                 410                 415

aaa att gcc aag aaa att tta tct aat gaa tta gta ata att gaa gta  1296
Lys Ile Ala Lys Lys Ile Leu Ser Asn Glu Leu Val Ile Ile Glu Val
            420                 425                 430

gga aaa aac aat aac ata aat ggc aaa caa ata gat gct aaa aaa cac  1344
Gly Lys Asn Asn Asn Ile Asn Gly Lys Gln Ile Asp Ala Lys Lys His
        435                 440                 445

ata cct tgg tta agt ata cag gtt att gta ttt act aca agt att cta  1392
Ile Pro Trp Leu Ser Ile Gln Val Ile Val Phe Thr Thr Ser Ile Leu
    450                 455                 460
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1407

tta ggt tgt att aag
Leu Gly Cys Ile Lys
465

<210> 7

<211> 469

<212> PRT

<213> Ehrlichia canis

<400> 7

Met Arg Asn Ile Leu Cys Tyr Thr Leu Ile Leu Ile Phe Phe Ser Phe
1 5 10 15

Asn Thr Tyr Ala Asn Asp Leu Asn Ile Asn Ile Lys Glu Ala Thr Thr
20 25 30

Lys Asn Lys Ile His Tyr Leu Tyr Val Glu His His Asn Leu Pro Thr
35 40 45

Ile Ser Leu Lys Phe Ala Phe Lys Lys Ala Gly Tyr Ala Tyr Asp Ala
50 55 60

Phe Asp Lys Gln Gly Leu Ala Tyr Phe Thr Ser Lys Ile Leu Asn Glu
65 70 75 80

Gly Ser Lys Asn Asn Tyr Ala Leu Ser Phe Ala Gln Gln Leu Glu Gly
85 90 95

Lys Gly Ile Asp Leu Lys Phe Asp Ile Asp Leu Asp Asn Phe Tyr Ile
100 105 110

Ser Leu Lys Thr Leu Ser Glu Asn Phe Glu Glu Ala Leu Val Leu Leu
115 120 125

Ser Asp Cys Ile Phe Asn Thr Val Thr Asp Gln Glu Ile Phe Asn Arg
130 135 140

Ile Ile Ala Glu Gln Ile Ala His Val Lys Ser Leu Tyr Ser Ala Pro
145 150 155 160

Glu Phe Ile Ala Thr Thr Glu Met Asn His Ala Ile Phe Lys Gly His
165 170 175

Pro Tyr Ser Asn Lys Val Tyr Gly Thr Leu Asn Thr Ile Asn Asn Ile
180 185 190

Asn Gln Glu Asp Val Ala Leu Tyr Ile Lys Asn Ser Phe Asp Lys Glu
195 200 205

Gln Ile Val Ile Ser Ala Ala Gly Asp Val Asp Pro Thr Gln Leu Ser
210 215 220

Asn Leu Leu Asp Lys Tyr Ile Leu Ser Lys Leu Pro Ser Gly Asn Asn
225 230 235 240

Lys Asn Thr Ile Pro Asp Thr Thr Val Asn Arg Glu Asp Thr Leu Leu
245 250 255

Tyr Val Gln Arg Asp Val Pro Gln Ser Val Ile Met Phe Ala Thr Asp
260 265 270

Thr Val Pro Tyr His Ser Lys Asp Tyr His Ala Ser Asn Leu Phe Asn
275 280 285

Thr Met Leu Gly Gly Leu Ser Leu Asn Ser Ile Leu Met Ile Glu Leu
290 295 300

Arg Asp Lys Leu Gly Leu Thr Tyr His Ser Ser Ser Ser Leu Ser Asn
305 310 315 320

Met Asn His Ser Asn Val Leu Phe Gly Thr Ile Phe Thr Asp Asn Thr
325 330 335

Thr Val Thr Lys Cys Ile Ser Val Leu Thr Asp Ile Ile Glu His Ile
340 345 350

Lys Lys Tyr Gly Val Asp Glu Asp Thr Phe Ala Ile Ala Lys Ser Ser
355 360 365

Ile Thr Asn Ser Phe Ile Leu Ser Met Leu Asn Asn Asn Asn Val Ser
370 375 380

Glu Ile Leu Leu Ser Leu Gln Leu His Asp Leu Asp Pro Ser Tyr Ile
385 390 395 400

Asn Lys Tyr Asn Ser Tyr Tyr Lys Ala Ile Thr Ile Glu Glu Val Asn
405 410 415

Lys Ile Ala Lys Lys Ile Leu Ser Asn Glu Leu Val Ile Ile Glu Val
420 425 430

Gly Lys Asn Asn Asn Ile Asn Gly Lys Gln Ile Asp Ala Lys Lys His
435 440 445

Ile Pro Trp Leu Ser Ile Gln Val Ile Val Phe Thr Thr Ser Ile Leu
450 455 460

Leu Gly Cys Ile Lys
465

<210> 8

<211> 675

<212> DNA

<213> Ehrlichia canis

<220>

<221> CDS

<222> (1)..(675)

<223> Protein translated from nucleotides 4,121 through 4,795 (ORF of unknown function).

<400> 8

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atg gta tta ttt atg aaa gct cat agc aca agt ata cgg aac ttt cag    48
Met Val Leu Phe Met Lys Ala His Ser Thr Ser Ile Arg Asn Phe Gln
  1               5                  10                  15

cct tta gaa aga gct gct ata atc att gca gtg tta ggt tta gct gca    96
Pro Leu Glu Arg Ala Ala Ile Ile Ile Ala Val Leu Gly Leu Ala Ala
            20                  25                  30

ttc ttg ttt gct gct gct gcc tgc agt gat cgt ttc caa aga ttg caa   144
Phe Leu Phe Ala Ala Ala Ala Cys Ser Asp Arg Phe Gln Arg Leu Gln
        35                  40                  45

tta aca aat cca ttt gta ata gca gga atg gtt ggc ctt gca gtt ctt   192
Leu Thr Asn Pro Phe Val Ile Ala Gly Met Val Gly Leu Ala Val Leu
    50                  55                  60

tta gtt gct tcc tta aca gca gca tta agt ata tgc tta act aaa agt   240
Leu Val Ala Ser Leu Thr Ala Ala Leu Ser Ile Cys Leu Thr Lys Ser
65                  70                  75                  80

aag caa gtc aca caa cat gct att aga cat cgc ttt gga tac gag tca   288
Lys Gln Val Thr Gln His Ala Ile Arg His Arg Phe Gly Tyr Glu Ser
                85                  90                  95

agc act tct tct tct gta ctg ctt gca ata tca ata att tct tta tta   336
Ser Thr Ser Ser Ser Val Leu Leu Ala Ile Ser Ile Ile Ser Leu Leu
            100                 105                 110

ctt gct gca gca ttt tgt gga aag ata atg ggt aat gac aac cca gat   384
Leu Ala Ala Ala Phe Cys Gly Lys Ile Met Gly Asn Asp Asn Pro Asp
        115                 120                 125

cta ttc ttt agc aag atg caa gaa ctc tcc aat cca ctt gtt gtt gca   432
Leu Phe Phe Ser Lys Met Gln Glu Leu Ser Asn Pro Leu Val Val Ala
    130                 135                 140

gct att gta gcc gtt tct gtt ttc cta ctc tca ttc gta atg tat gct   480
Ala Ile Val Ala Val Ser Val Phe Leu Leu Ser Phe Val Met Tyr Ala
145                 150                 155                 160

gca aag aac att ata agt cca gat aaa caa act cac gtt att ata tta   528
Ala Lys Asn Ile Ile Ser Pro Asp Lys Gln Thr His Val Ile Ile Leu
                165                 170                 175
```

```
tct aat caa caa act ata gaa gaa gca aaa gta gat caa gga atg aat   576
Ser Asn Gln Gln Thr Ile Glu Glu Ala Lys Val Asp Gln Gly Met Asn
            180                 185                 190

att ttg tca gca gta ctc cca gca gct ggc att gac atc atg act ata   624
Ile Leu Ser Ala Val Leu Pro Ala Ala Gly Ile Asp Ile Met Thr Ile
        195                 200                 205

gct tct tgt gac att tta gca gtg agc agc cgg gga tcc tct cag cat   672
Ala Ser Cys Asp Ile Leu Ala Val Ser Ser Arg Gly Ser Ser Gln His
    210                 215                 220

                                                                  675
caa
Gln
225
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<210> 9

<211> 225

<212> PRT

<213> Ehrlichia canis

<400> 9

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Met Val Leu Phe Met Lys Ala His Ser Thr Ser Ile Arg Asn Phe Gln
  1               5                  10                  15

Pro Leu Glu Arg Ala Ala Ile Ile Ile Ala Val Leu Gly Leu Ala Ala
            20                  25                  30

Phe Leu Phe Ala Ala Ala Ala Cys Ser Asp Arg Phe Gln Arg Leu Gln
        35                  40                  45

Leu Thr Asn Pro Phe Val Ile Ala Gly Met Val Gly Leu Ala Val Leu
    50                  55                  60

Leu Val Ala Ser Leu Thr Ala Ala Leu Ser Ile Cys Leu Thr Lys Ser
65                  70                  75                  80

Lys Gln Val Thr Gln His Ala Ile Arg His Arg Phe Gly Tyr Glu Ser
                85                  90                  95

Ser Thr Ser Ser Ser Val Leu Leu Ala Ile Ser Ile Ile Ser Leu Leu
            100                 105                 110

Leu Ala Ala Ala Phe Cys Gly Lys Ile Met Gly Asn Asp Asn Pro Asp
        115                 120                 125

Leu Phe Phe Ser Lys Met Gln Glu Leu Ser Asn Pro Leu Val Val Ala
    130                 135                 140

Ala Ile Val Ala Val Ser Val Phe Leu Leu Ser Phe Val Met Tyr Ala
145                 150                 155                 160
```

Ala Lys Asn Ile Ile Ser Pro Asp Lys Gln Thr His Val Ile Ile Leu
165 170 175

Ser Asn Gln Gln Thr Ile Glu Glu Ala Lys Val Asp Gln Gly Met Asn
180 185 190

Ile Leu Ser Ala Val Leu Pro Ala Ala Gly Ile Asp Ile Met Thr Ile
195 200 205

Ala Ser Cys Asp Ile Leu Ala Val Ser Ser Arg Gly Ser Ser Gln His
210 215 220

Gln
225

<210> 10

<211> 417

<212> DNA

<213> Ehrlichia canis

<220>

<221> CDS

<222> (1)..(417)

<223> Protein translated from complementary sequence derived from nucleotides 4,884 to 5,300 (partial lipoprotein signal peptidase homolog).

<400> 10

gat cag gta agt aaa tgg tat gta gta aat ttg ata gga gat aaa ggt 48
Asp Gln Val Ser Lys Trp Tyr Val Val Asn Leu Ile Gly Asp Lys Gly
1 5 10 15

gta ata gag ata tta agc ttc ttg cgc ttt act aca gtg tgg aat gct 96
Val Ile Glu Ile Leu Ser Phe Leu Arg Phe Thr Thr Val Trp Asn Ala
20 25 30

gga att agt ttt ggt ata tta aat aac ttt gaa tat agt aat gtt gtt 144
Gly Ile Ser Phe Gly Ile Leu Asn Asn Phe Glu Tyr Ser Asn Val Val
35 40 45

ttt tgt agt atc tcg att ttg att act tgt gtt tta tgc tac tta ttt 192
Phe Cys Ser Ile Ser Ile Leu Ile Thr Cys Val Leu Cys Tyr Leu Phe
50 55 60

ata gta cag cca cat tat aga tta cct ctt gta atc att att ggg ggg 240
Ile Val Gln Pro His Tyr Arg Leu Pro Leu Val Ile Ile Ile Gly Gly
65 70 75 80

```
tca ata gga aat atc ata gat aga ata aga tat ggt gct gtc tat gat   288
Ser Ile Gly Asn Ile Ile Asp Arg Ile Arg Tyr Gly Ala Val Tyr Asp
                85                  90                  95

ttt ata gat ttt tat atc aat aac tta cat tgg cct gta ttc aac ctg   336
Phe Ile Asp Phe Tyr Ile Asn Asn Leu His Trp Pro Val Phe Asn Leu
            100                 105                 110

gcg gat tct ttt ata ttt tta ggt ata gta ata ata atg gca aag agt   384
Ala Asp Ser Phe Ile Phe Leu Gly Ile Val Ile Ile Met Ala Lys Ser
        115                 120                 125

aat aac cac atg aaa caa att aac tgt aac tcc                       417
Asn Asn His Met Lys Gln Ile Asn Cys Asn Ser
    130                 135
```

<210> 11

<211> 139

<212> PRT

<213> Ehrlichia canis

<400> 11

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Asp Gln Val Ser Lys Trp Tyr Val Val Asn Leu Ile Gly Asp Lys Gly
  1               5                  10                  15

Val Ile Glu Ile Leu Ser Phe Leu Arg Phe Thr Thr Val Trp Asn Ala
            20                  25                  30

Gly Ile Ser Phe Gly Ile Leu Asn Asn Phe Glu Tyr Ser Asn Val Val
        35                  40                  45

Phe Cys Ser Ile Ser Ile Leu Ile Thr Cys Val Leu Cys Tyr Leu Phe
    50                  55                  60

Ile Val Gln Pro His Tyr Arg Leu Pro Leu Val Ile Ile Ile Gly Gly
65                  70                  75                  80

Ser Ile Gly Asn Ile Ile Asp Arg Ile Arg Tyr Gly Ala Val Tyr Asp
                85                  90                  95

Phe Ile Asp Phe Tyr Ile Asn Asn Leu His Trp Pro Val Phe Asn Leu
            100                 105                 110

Ala Asp Ser Phe Ile Phe Leu Gly Ile Val Ile Ile Met Ala Lys Ser
        115                 120                 125

Asn Asn His Met Lys Gln Ile Asn Cys Asn Ser
    130                 135
```